Methods: Coronary flow velocimetry (Doppler-tipped guidewire) and quantitative angiography were performed in a non-stenotic artery at control (Con), and during a maximally dilating dose of intracoronary adenosine before and after nitroglycerin (NTG) 100 μg i.c. in 6 patients. CFR was measured conventionally using peak/rest flow velocity changes alone (Vel) and flow estimates incorporating angiographic vessel cross-sectional area (VxA).

Results: (mean ± s.e.m.)

Coronary Diameter (mm)			CFR		
Con	adenosine	NTG	Con Vel	Con VxA	NTG Vel
2.50 ± 0.26	2.89 ± 0.30*	2.93 ± 0.28*	3.0 ± 0.2	4.0 ± 0.3†	3.8 ± 0.4‡

*p < 0.01 vs control, †p < 0.01 vs con Vel, ‡p = 0.08 vs con Vel

Conclusions: 1) Intracoronary adenosine produces concurrent conduit as well as resistance vessel dilation that is similar in magnitude to nitroglycerin. 2) Coronary flow reserve is underestimated if blood flow changes are assessed using flow velocity measurements alone. 3) Coronary flow reserve measured using velocity changes after pretreatment with nitroglycerin is similar to measurement that incorporates angiographic cross-sectional area, and may obviate the need for area determination.

995-18 Coronary Endothelial Dysfunction in Patients with Normal Coronary Angiography and Abnormal Functional Tests

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Coronary endothelial dysfunction is characterized by a vasoconstrictive response to the endothelial-dependent vasodilator acetylcholine. In patients with normal coronary angiograms, stress-induced myocardial ischemia has been considered to be false-positive on functional testing. Since coronary vasodilatation during exercise is endothelial-dependent, this study was designed to test the hypothesis that patients with stress-induced myocardial ischemia and normal coronary angiograms may demonstrate coronary endothelial-dependent vasoconstriction to acetylcholine. Thus, coronary angiography with graded doses of intracoronary acetylcholine was performed in 30 patients who had undergone previous stress functional tests (14 exercise thallium, eight exercise echocardiography, four dipyridamole thallium, and four dobutamine echocardiography). Twenty patients had an abnormal functional test (Group 1), and 10 patients had normal functional tests serve as a control group (Group 2).

Acetylcholine	Myocardial Ischemia by Functional Testing	
	Positive (Group 1)	Negative (Group 2)
Vasoconstriction	19	0
Vasodilation	1	10
TOTAL	20	10

$p < 0.001$

This study demonstrates that patients with stress-induced myocardial ischemia and normal coronary angiograms represent coronary endothelial dysfunction.

995-19 The Impact of Mental Stress and α-Adrenergic Activation on the Microcirculation of Patients with Coronary Artery Disease

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Mental stress results in sympathetic activation and provokes constriction of diseased epicardial coronary artery segments, which may decrease coronary blood flow. We investigated the effect of mental stress (10 min. video game) on the coronary microcirculation by measuring cardiac norepinephrine spillover (NESP) and left anterior descending (LAD) coronary artery blood flow (intracoronary Doppler flow velocity and quantitative angiography) in non-significantly diseased (<40% stenosis) LAD arteries of 7 patients with significant coronary artery disease (CAD; >50% stenosis) of other vessels. These responses were compared to those in 5 patients with normal coronary angiograms (NCA). Coronary vascular resistance (CVR) was calculated by dividing mean blood pressure (MBP) by coronary blood flow. Measurements during mental stress were compared to pre-mental stress measurements. Data = mean ± S.D. during mental stress.

%Change in:	MBP	NESP	LAD diameter	CVR
NCA	+13 ± 8	+123 ± 130	+4 ± 10	−26 ± 9
CAD	+19 ± 10	+90 ± 64	+1 ± 6	−4 ± 18*

*p < 0.05 vs NCA

Mental stress resulted in significant coronary microvascular dilation in patients with NCA ($p < 0.05$ vs pre-mental stress) in contrast to no vasodilation in patients with CAD (p = NS). 5 CAD patients underwent repeat mental stress (MS), following intracoronary phentolamine (P) 0.02 mg/kg.

%Change in:	MBP	NESP	LAD diameter	CVR
Control MS	+26 ± 13	+87 ± 40	+1 ± 6	−9 ± 14
P + MS	+18 ± 9	+254 ± 117**	+3 ± 7**	−28 ± 19**

**p < 0.01 vs Control MS

Even though phentolamine increased NESP substantially due to presynaptic α_2-adrenergic blockade, microvascular dilation was greater during repeat mental stress in CAD patients than during their initial mental stress, and similar in magnitude to the response of NCA patients. Thus, coronary atherosclerosis is associated with absence of a microvascular vasodilator response to mental stress, due to α-adrenergic constrictor effects on the coronary microcirculation.

995-20 Effect of Hypoxia on Coronary Blood Flow and Myocardial Oxygen Extraction in Patients with Coronary Artery Disease

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Previous studies in healthy subjects have shown that, at rest, the myocardium responds to a reduced arterial oxygen (O_2) content with an increased extraction of O_2 from the coronary blood rather than with an increased coronary blood flow (CBF). The effect of controlled hypoxia on CBF, coronary sinus blood O_2 saturation (CS-O_2%) and O_2 delivery (MVO_2) was investigated in 7 anginal patients (all men, mean age 54 ± 11 yrs) with at least one critical coronary stenosis (>50% reduction in internal lumen diameter) of the left coronary artery. Patients breathed through a face mask 10% O_2 in nitrogen and arterial O_2 saturation (A-O_2%) was monitored by a finger-piece oxymeter. CBF was monitored by using an 8F Doppler catheter positioned in the left coronary ostium. CS-O_2% was obtained by blood sampling from the coronary sinus. Twelve-lead ECG and arterial blood pressure were continuously monitored throughout the procedure. The ratio between rate-pressure product (RPP) and MVO_2 was used as an index of the matching between O_2 supply and O_2 demand. The results are summarized in the following table.

A-O_2 % (%)	CBF (ml/min)	CS-O_2 % (ml%)	RPP (bpm × mmHg)	RPP/MVO_2 (bpm × mmHg × min/ml)
Basal	128 ± 38	34 ± 5	8812 ± 2200	795 ± 655
90	145 ± 56	33 ± 8	9607 ± 2411	736 ± 599
85	173 ± 57*	30 ± 4	10530 ± 2561*	748 ± 757
80	191 ± 77*	29 ± 6	10982 ± 2956*	790 ± 831

*p < 0.05 vs basal

Thus, in patients with coronary artery disease, differently from normal subjects, myocardial hypoxia is compensated for by an increase of CBF rather than by an increase of myocardial O_2 extraction. These findings suggest that coronary atherosclerosis may limit the capacity of the myocardium to extract O_2.

995-21 Coronary Flow Reserve Does Not Improve After Successful Rotablator Atherectomy and Adjunctive Angioplasty

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Although lumen enlargement after coronary balloon angioplasty (PTCA) is associated with improvement in coronary flow velocity (CFV) and coronary flow reserve (CFR), Rotablator atherectomy (MRA) results in lumen enlargement and distal microembolization, which may attenuate coronary blood flow and CFR. Accordingly, spectral flow velocities were obtained with Doppler Flowires in 16 arteries (9 LAD, 3 LCX, 4 RCA in 16 patients, [age 62 ± 12 yrs]) before, after MRA and after adjunctive PTCA. Basal (BCFV) and peak hyperemic flow velocities (PCFV) after intracoronary adenosine (10–30 μg) were recorded in identical angiographic positions. Blood pressure and heart rate were constant between measurements. Diameter stenosis (DS) was determined by quantitative coronary angiography.

	Pre-Intervention	Post MRA	Post PTCA
BCFV (cm/s)	16.3 ± 6.6	25.0 ± 13.4*	36.8 ± 13.9*
PCFV (cm/s)	21.1 ± 11.1	33.7 ± 18.7*	48.9 ± 24.8*
CFR	1.28 ± 0.31	1.20 ± 0.36†	1.44 ± 0.38†
DS (%)	63 ± 17	49 ± 18**	28 ± 12**

*p < 0.0001, **p < 0.01, †p = NS, compared with baseline (ANOVA)

Conclusions 1) MRA and adjunctive PTCA result in significant increases in coronary lumen dimensions, basal flow velocity and hyperemic peak coronary flow velocity. 2) Failure to increase coronary flow reserve after MRA and adjunctive PTCA is due to proportional increases in basal and hyperemic flow velocity and not to impaired coronary artery flow from microembolization.

995-22 The Effect of Perioperative Storage Solutions on the Long Term Vein Graft Function and Morphology

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It has been shown that suboptimal preparation of a vein graft prior to its insertion results in immediate morphological and functional damage to the endothelial cells but not to the underlying smooth muscle cells. However, little is known if such perioperative injury to the vein grafts may influence the subsequent development of intimal hyperplasia and smooth muscle cell contractility. This study examines the influence of storage in saline or Ringer's lactate on the development of intimal hyperplasia and vasomotor function in experimental vein grafts. Twenty-six NZW rabbits had a carotid vein bypass graft performed after the veins had been immersed (15 minutes) in either heparinized saline (Sal; n = 13) or Ringer's lactate (RL; n = 13) and each group was harvested after 28 days for either histology (n = 8) or functional studies (n = 5; four 5 mm rings/graft). Saline storage of the vein graft resulted in a 38% increase in the thickness of the intimal hyperplasia (113 ± 2 vs. 83 ± 2 μm, Sal vs. RL; mean ± SEM, $p < 0.05$) without a change in medial thickness (87 ± 5 vs. 86 ± 8 μm, Sal vs. RL; $p > 0.05$). There was no difference in the sensitivity to norepinephrine, serotonin and bradykinin between the two sets of vein grafts. The maximal contractile forces to serotonin and bradykinin were increased in the saline compared to Ringer's lactate stored vein grafts.

	Saline	Ringer's	p-value
Norepinephrine	0.88 ± 0.12	1.57 ± 0.20	<0.05
Serotonin	1.23 ± 0.15	0.37 ± 0.13	<0.01
Bradykinin	2.08 ± 0.11	0.52 ± 0.07	<0.01

Values are the standardized maximal contractile force (maximal contraction / contraction to 60 mM KCl) expressed as mean ± SEM.

Saline storage of the vein graft results in the increased development of intimal hyperplasia with an overall enhanced contractility but without changes in agonist sensitivity. This study places further emphasis on the need for good perioperative care of the vein bypass graft because it results not only in the previously documented short term problems but also in long term structural and contractile changes which may contribute to decreased graft patency.

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Wednesday, March 22, 1995, 9:00 a.m.–11:00 a.m.
Ernest N. Morial Convention Center, Hall E
Presentation Hour: 9:00 a.m.–10:00 a.m.

996-8 In Vivo Adenovirus-Mediated Gene Transfer via the Pulmonary Artery of Rats

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Gene transfer into the pulmonary vasculature may be a powerful technique both for the investigation of pulmonary pathophysiology and for the development of genetic therapies for pulmonary vascular disease. To evaluate the potential for in vivo pulmonary arterial gene transfer, we infused adenoviral vectors into the left pulmonary artery of Sprague-Dawley and cotton rats. Access to the left pulmonary artery was obtained either by a percutaneous, transcatheter approach, or via a thoracotomy and pulmonary arteriotomy. When using the thoracotomy approach, both the pulmonary arterial inflow and the pulmonary venous outflow were occluded during vector infusion and throughout a subsequent 20 minute dwell period. The success of gene transfer was assessed by staining for evidence of recombinant gene expression in lungs excised at time points ranging from 48 to 72 hours after virus infusion. *Results:* Using the surgical technique, pulmonary gene transfer was successful in 15% of surviving Sprague-Dawley and 30% of surviving cotton rats. Percutaneous pulmonary gene transfer was not successful. In those rats with pulmonary gene transfer, 1–8% of total pulmonary cells expressed the recombinant gene. Recombinant gene expression was found in endothelial cells (0.2–18% of total transduced cells), smooth muscle cells (0–3%), macrophages (1–7%), airway epithelial cells (2–50%), and alveolar epithelial cells (38–94%). Studies investigating the low rate of successful gene transfer in individual animals suggested that insufficient physical contact of the virions with pulmonary cells was the likely etiology. *Conclusion:* In vivo gene transfer into the rat pulmonary vasculature can be accomplished with adenovirus vectors. Pulmonary arterial infusion of the vectors results in low level endothelial cell transduction, with higher levels of gene transfer into nonvascular pulmonary cells.

996-9 Immune-mediated Response to Adenovirus Affects the Expression of Genes Delivered to Adult Rat Myocardium

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Direct injection of adenovirus (Ad) has been suggested as an efficient method for *in vivo* gene transfer into the myocardium and as a potential tool for gene therapy of myocardial disease. However, previous studies demonstrated that this method is limited by a sharp decline of gene expression after 1 week. To test the hypothesis that an immune-effector mechanism is involved in this decline we compared the results following injection of Ad-5 carrying the β-galactosidase(β-gal) gene into the left ventricular myocardium of athymic nude (NR; n = 7) versus Sprague-Dawley (SD; n = 7) rats. Volumes of 25–50 μl of a 1.0 × 10^9 PFU/ml solution were injected. Hearts were harvested and stained for β-gal activity at 30–35 days. β-gal activity was scored on a 1– 4+ rating system based on the number of stained cells observed per high power field (hpf).

	4+ >50 Cells/hpf	3+ >25 Cells/hpf	2+ >10 Cells/hpf	1+ 0–5 Cells/hpf
NR n = 7	5	0	2	0
SD n = 7	0	0	0	7

Score 4+ was significantly more frequent among NR vs. SD hearts (5 of 7 vs. 0 of 7; $p = 0.02$). Further, an inflammatory response was limited to the epicardium in NR as compared to SD in which there was also an intense inflammation with mononuclear cell infiltration and collagen deposition in the myocardium. The present model provides efficient gene expression for at least 35 days without significant inflammatory reaction. Our data suggests that an immune-mediated response to Ad can severely effect the expression of genes delivered by this virus.

996-10 A Novel Approach to Identifying mRNAs Differentially Expressed Under Hemodynamic Pressure Overload During Sheep Fetal Heart Development

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The adaptive response to pressure overload is different in neonatal hearts with congenital lesions than in adult hearts with acquired lesions. In adult hearts this response is characterized by an altered pattern of gene expression. Little is known about the changes in gene expression that occur in neonates. We have used a novel approach to examine these changes in expression of messenger RNAs in an *in vivo* model of fetal lambs heart after induced acute pressure overload. In ewes (which normally carry twin gestations) the main pulmonary artery of one fetal lamb was banded *in utero* for one hour to produce an acute pressure overload on the right ventricle (RV) while the twin underwent a sham operation. Total RNA was isolated and reverse transcribed from both the banded and control RV. The resulting cDNAs were amplified by the polymerase chain reaction technique using generalized oligonucleotide primers and the products displayed on sequencing gels. Comparative analysis of the differentially expressed cDNA bands from each condition revealed both up-regulation and down-regulation of several cardiac genes in the banded fetal hearts compared with the control lamb hearts. No morphological changes in the cardiac tissues were observed during this same time period. Eighty (300–500bp) differentially expressed cDNAs were identified. Twenty-three of these were analyzed by Northern blot and four were confirmed as being differentially expressed. Two of these were sequenced, identifying the 3'-untranslated regions which had no identifiable homology to the sequences in GENEBANK. This study reports on using an *in vivo* fetal lamb model to study changes in gene expression induced by hemodynamic pressure overload using a technique that identifies all differentially expressed messages.

Conclusion: This is the first study to examine alterations in fetal cardiac gene expression to hemodynamic overload in a large mammalian model.